attachment to the *Scorpions* primer or incorporation via phosphoramidite (solid phase) chemistry.

FRET embodiment

In this modification of the basic system, the dyes involved form an energy transfer pair. One of the dyes is positioned close to the 3' end of the target binding region, while the other is placed close to the 3' terminus of the amplicon binding region (see Figure 5a,b). The probe must hybridise very close to the primer thus bringing together the FRET pair and producing an enhanced fluorescence signal

No-quencher embodiment

A fluorophore is attached to the tail of the *Scorpions* primer (see Figure 6 (a),(b) & (c)). Random folding of the *Scorpions* primer around the fluorophore provides sufficient quenching of the fluorophore. We believe this may be due principally to the nucleotide, guanylic acid. In order to maximise quenching it is preferred, but not essential, to have the fluorophore at or around the middle of the primer, with sufficient additional DNA to quench efficiently. Quenching efficiency is dependent on the sequence of the surrounding DNA. Binding of the target binding region of the tail to the target region alters the conformation of the DNA sufficiently to remove this quenching. We prefer this embodiment as an amplicon detector.

Bimolecular embodiment

The fluorophore and quencher may be introduced on two separate but complementary molecules (Figure 7a). The fluorophore and quencher may be on either end of the probe or complementary strands, provided that hybridisation of the two strands brings the fluorophore/quencher pair into close proximity. After a round of denaturation, annealing and extension, the fluorophore remains quenched, as the bimolecular moiety re-forms (Figure 7b). The non *Scorpion*, free strand is in excess to ensure that this bimolecular interaction occurs and for this reason it is preferred that this molecule carries the quencher, to minimise backgrounds. However, after a further round of denaturation and annealing, the self-probing strand forms (Figure 7c) and the free quencher (oligo) is unable to compete with this event kinetically or thermodynamically thus leading to an increase in fluorescence.

If required one of the molecules may comprise a secondary structure such as a hairpin structure so as to allow the attachment of for example more quencher species for more efficient quenching of a fluorophore on the other molecule.

Capture Probe embodiment

In addition to the embodiments discussed above, amplicons may be specifically captured and probed using the same non-amplifiable tail (see Figures 8a & 8b). In a further specific embodiment the capture and tail sequences are provided as non-contiguous features ie. together with the template binding region they form a branched primer structure (cf, Figure 8c). After amplification the amplicon may be captured onto a solid surface, whilst the signal generation remains amplicon specific. Alternatively the capture sequences and signalling system may be on opposite ends of the amplicons. In this way, generic "chips" with the same capture sequences may be used to analyse many different targets- the capture regions remain unchanged while the amplifier and probe elements vary.

Stem embodiment

In this embodiment the primer tail comprises self complementary stems (also DNA, RNA, 2'-O-methyl RNA, PNA and their variants) which flank the amplicon binding region and which carry a fluorophore quencher pair, such that hairpin formation by the two stems brings the F/Q pair together causing the fluorescence to be substantially quenched ("off"). The fluorophore and quencher can be placed on either arm, depending upon preference or synthetic simplicity; we prefer to have the quencher on the 3' arm (ie adjacent to the blocker in the middle of the molecule)].

At high temperatures, the stem duplex is disrupted and the fluorophore is unquenched (ie "on"- Figure 9a); at lower temperatures, however, the stem duplex forms and the fluorescence is substantially off (Figure 9b).

In an amplification cycle

After initial denaturation, annealing and extension, the *Scorpions* amplicon comprises a region complementary to the loop region at its 5'-end (Figure 10a). Upon a second round of denaturation (Figure 10b) and annealing, the tail hybridises to the newly synthesised region with great efficiency (a unimolecular interaction) and fluorescence remains unquenched (Figure 10c). Unextended primers, however, will continue to form their quenched

conformation. Meanwhile, the "reverse" primer will have hybridised to this same strand and synthesis goes on. We believe that the tail is (at least partially) displaced by the Taq polymerase and the remainder melts off easily since the probes are short. At this stage, the Taq polymerase completes the synthesis of this strand until it encounters the amplification blocker. Because signals are strong and the priming function is identical to the non-Scorpions variant, not all the primer needs to be in the *Scorpions* form. Indeed, we have obtained strong signals when 10% or less of the primer was in the *Scorpions* form. This allows cheaper reactions and also permits the balancing of signal strengths where two different fluorophores are used.

The *Scorpions* primers of the invention may be used in place of conventional amplification primers, such as PCR primers and are not expected to interfere with their amplification function. In a two-tube ARMS test (normal and mutant) the *Scorpions* primer may conveniently be the common primer (Figure 11a), with the production of signal dependent upon ARMS amplification.

However, it is equally viable to place the signalling entity on the ARMS primers. Each ARMS primer may be labelled with different fluorochromes (F1, F2), thus permitting single tube genotyping (STG)- that is both reactions are run in the same tube and the amplicons are distinguished by their characteristic "colour" (Figure 11b). Alternatively, the signalling entity may carry the allelic specificity (see Example 2): the primers are standard (non-ARMS) primers and two different probe sequences to match the two allelic variants are introduced on two variants of one of the primers (Figure 12a, b). It has been found that probes which can form hairpins in the absence of target are better discriminators of single base mismatches than the untailed versions of the same probes. In another manifestation, probes for each variant may be introduced one each on the two amplimers (Figure 12c, d) thereby probing different strands of the reaction. Finally, combinations of these ideas are possible: one subset of *Scorpions* primers may be used for allele discrimination, while other primers in the same mix may act as control probes to detect the amplicon itself (Figure 12e). Discrimination between these events is achieved either by fluorescence wavelength or alternatively by the use of probe elements having the same fluorophore but different T_ms which may then be discerned by measuring the fluorescence over a temperature range.